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# Shorter Notes

**Mericlinal Chimeras in the Gametophyte of *Dryopteris thelypteris* (L.) Gray.**—The typical fern gametophyte is a heart-shaped monolayer of cells with an apical cell as the meristem. All cells are derived from an apical cell lineage with each consecutive apical cell dividing into a daughter apical cell and a vegetative cell with the latter proliferating into a group of cells that Douin (Rev Gen. Bot. 38:487–508. 1924) termed a merophyte (Fig. 1); Gifford (Ann. Rev. Plant Physiol. 34:419–440. 1983) specifically notes that a merophyte is to include the original sister cell as well as all its derivatives. Later Korn (Acta Biotheoretica 41:175–189. 1993) described a merophyte as a clone. Klekowski (Evolution 38: 417–426. 1984) raised the question of whether an apical-celled organ can express a chimera by noting that a mutation in the apical cell will lead to all subsequent cells as mutant and therefore a persistent mericlinal chimera is not possible. A mericlinal chimera in angiosperms includes a region of one layer within either L1, L2 or L3 and in a fern gametophyte it would include one transient subclone of a merophyte clone. The question is then can chimeras occur in fern gametophytes? This note reports a mutant of the fern *Dryopteris thelypteris* (L.) Gray that produces not one but a number of mericlinal chlorophyll chimeras in a gametophyte, that is, it appears to be an eversporting chimera (Hejnowicz , Recent Adv. Bot. 2:146–148. 1951).

Sterilized spores were plated in agar dishes and periodically observed for morphology of subsequent gametophytes (Korn, Bot. J. Linn. Soc. 68:63–171.1974). Merophytes can be easily identified in *D. thelypteris* as a papilla forms on the most anterior, marginal cell of each merophyte (Korn, Bot. J. Linn. Soc. 68: 63–171. 1974) (Figs. 1 and 2). About 1,100 spores were seeded in each of three plates and 16 chimeric gametophytes (Fig. 3) were found for a frequency of 16/3300, or 0.0048, gametophytes with chimeras.

Where in the parental sporophyte the mutation occurred can be calculated as follows. First, the frequency of gametophytes or spores with the mutation of 0.0048 is converted to the frequency heterozygous diploid cells of the leaf, or 0.0048 becomes 0.0096, or approximately 0.01. Next, the fraction of a leaf with the patch of heterozygous cells is determined. Each sporangium has about 22.7 $\pm$ 3.3 (n = 25) spores which come from 22.7/4, or about 5.7 sporocytes, a sorus has an average of 30.6 $\pm$ 6.0 (n=25) sporangia per sorus, a secondary pinnae has an average of 12.6 $\pm$ 1.9 (n = 25) sori, a primary pinna has 45.8 $\pm$ 6.3 secondary pinnae and a leaf has 45.7 $\pm$ 9.5 (n= 5) primary pinnae for a total count of 5.7 $\times$ 30.6 $\times$ 12.6 $\times$ 45.8 $\times$ 45.7, or there are about 4,599,901 sporocytes per leaf. If 0.01 sporocytes are heterozygous then there were 4,599,901 $\times$ 0.01, or 45,999 heterozygous sporocytes per leaf. With about 45,999 heterozygous sporocytes per leaf and with 5.7 $\times$ 30.6 $\times$ 12.6, or 2,198 sporocytes per secondary pinna, there were 45,999/2,198, or 20.9 secondary pinna with heterozgygous sporocytes. With 45.8 secondary pinnae per primary

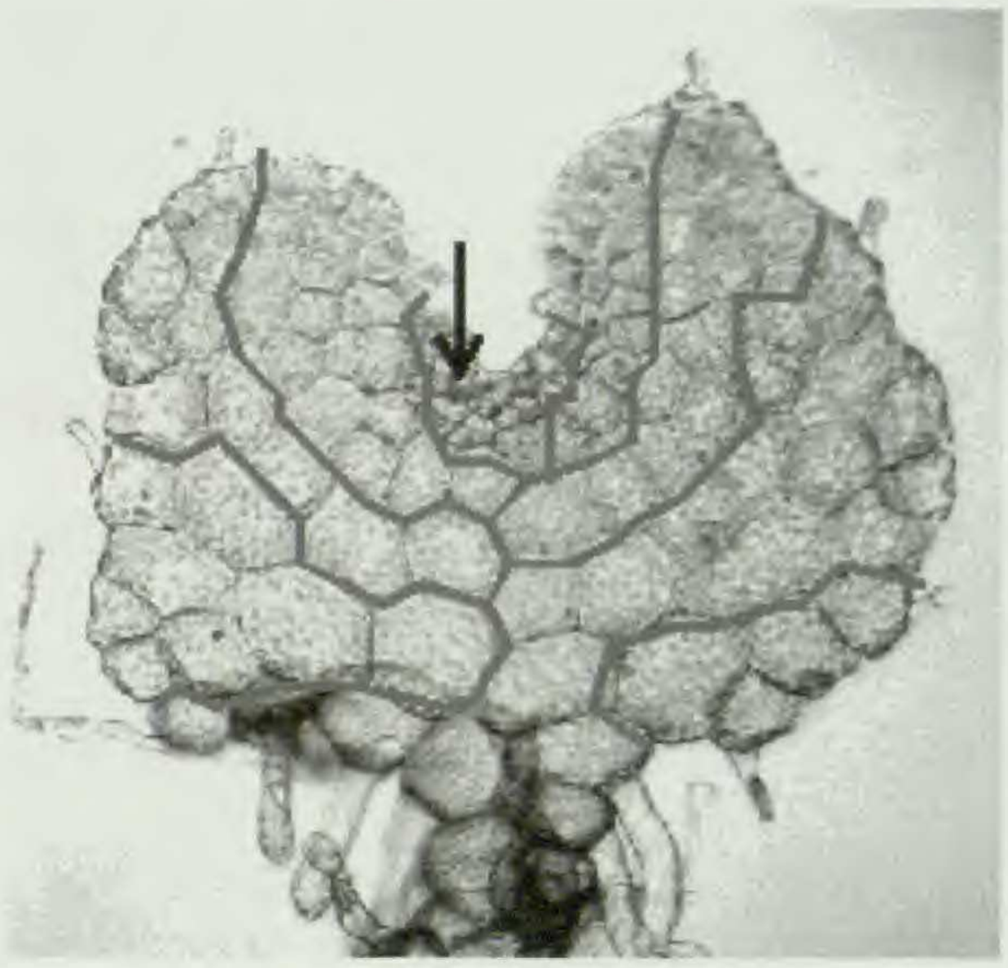

FIG. 1. Wild type gametophyte with merophyte boundaries drawn in red and arrow points to location of the apical cell.

pinnae then 20.9/45.8, or about a region of 46% of a primary pinna, or (1/45.7) × 0.46 or 0.01 of leaf area had this mutation. Third, the distributions of sporocytes in a sporangium, sporangia in a sorus, sori in a secondary pinna, secondary pinnae in a primary pinna and primary pinnae in a leaf are regular, namely, ordered, which permits fractions of leaf area to be given in any of these units, such as 0.01 of a leaf based on sporocyte calculations.

The above calculations are based on the assumption that there was a single mutation in some leaf that was passed on to spores from that portion of the leaf where the mutation occurred. It is also possible that the entire leaf and all other leaves of the mother sporophyte were heterozygous because one of two possible gametophytic parents was mutant. This is an unlikely explanation because gametophytes studied here came from only one leaf while spores from over eight other leaves from this sporophyte over a period of ten years never produced chimeric gametophytes.

Three gametophytes where all clones could be clearly recognized were analyzed in detail for the number of cells per albino subclone (Fig. 4) with the lowest value of one cell as the most frequent (20) and the largest subclone of 35 cells as the most infrequent (1). The data set on number of cells in a subclone was tested to see if it follows a geometric series, $P(n) = Nq^{n-1}$p (Meyer, Introductory Probability and Statistical Applications, Addison Wesley. 1965), where $N$ is the number of merophytes scored in the observed data, or 58, $n$ is

FIG. 2. A marginal papillar cell.

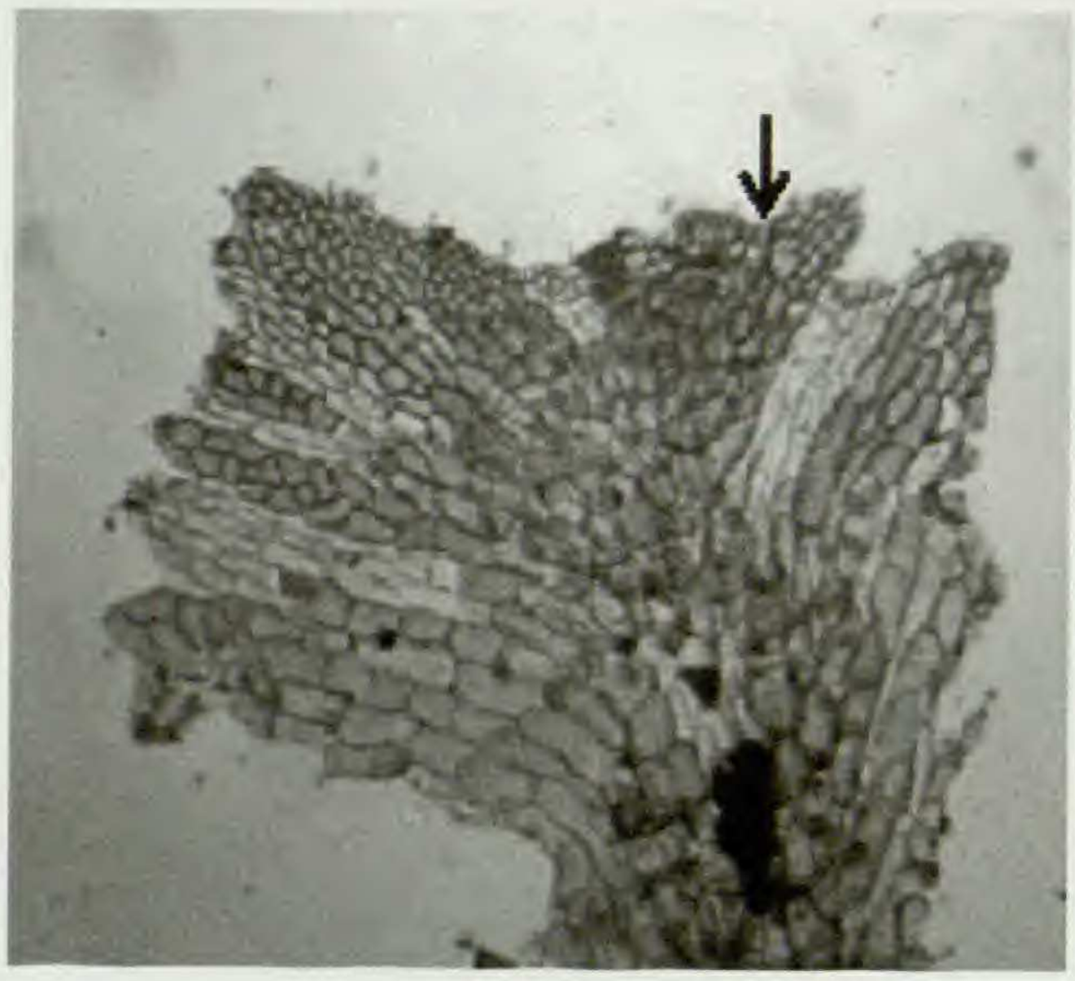

FIG. 3. Mutant gametophyte with various sizes of albino subclones and arrow points to apical cell.

the number of cell cycles, or cell generations, $q$ is the probability of subclonal cells dividing at $n^{th}$ generation and $q$ is the probability that subclonal cells cease dividing at the $n^{th}$ generation (Fig. 4). The value of $q$ was determined by averaging the ratios of $n/n+1$ and $p$ is 1-q. A $\chi^2$ probability of this equation generating data like the observed is >90%. The good fit of expected to obtained data indicates albino clones arise at any time during merophyte formation, more and smaller subclones form as a merophyte increases in cell number.

A second study of these 58 merophytes was to determine the number of albino subclones per merophyte (Fig. 5). The data has a sharp peak at two and does not follow any tested probability distribution. It is assumed that an early subclone proliferates and therefore limits the number of more, small subclones later in a merophyte. A computer program was written whereby there were 58 merophyte inital cells and the probability of an albino subclone forming is

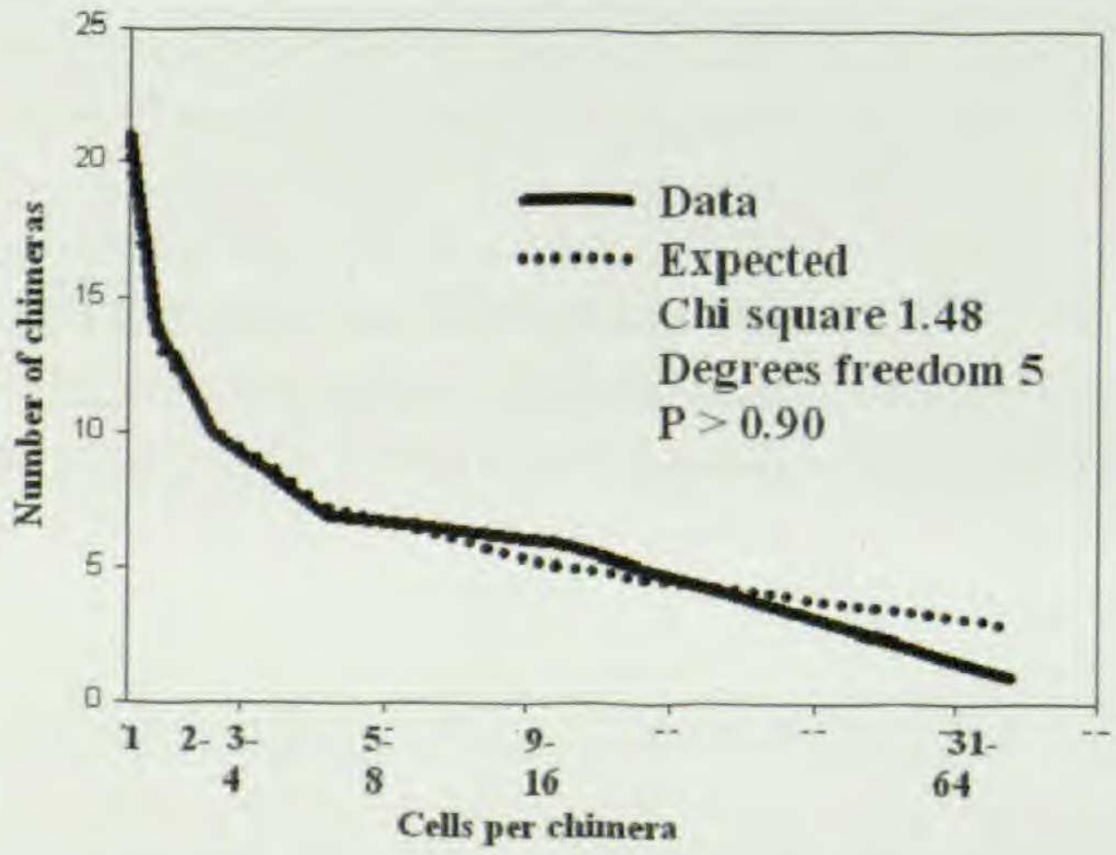


FIG. 4. Cells per albino subclone vs number of such cases.

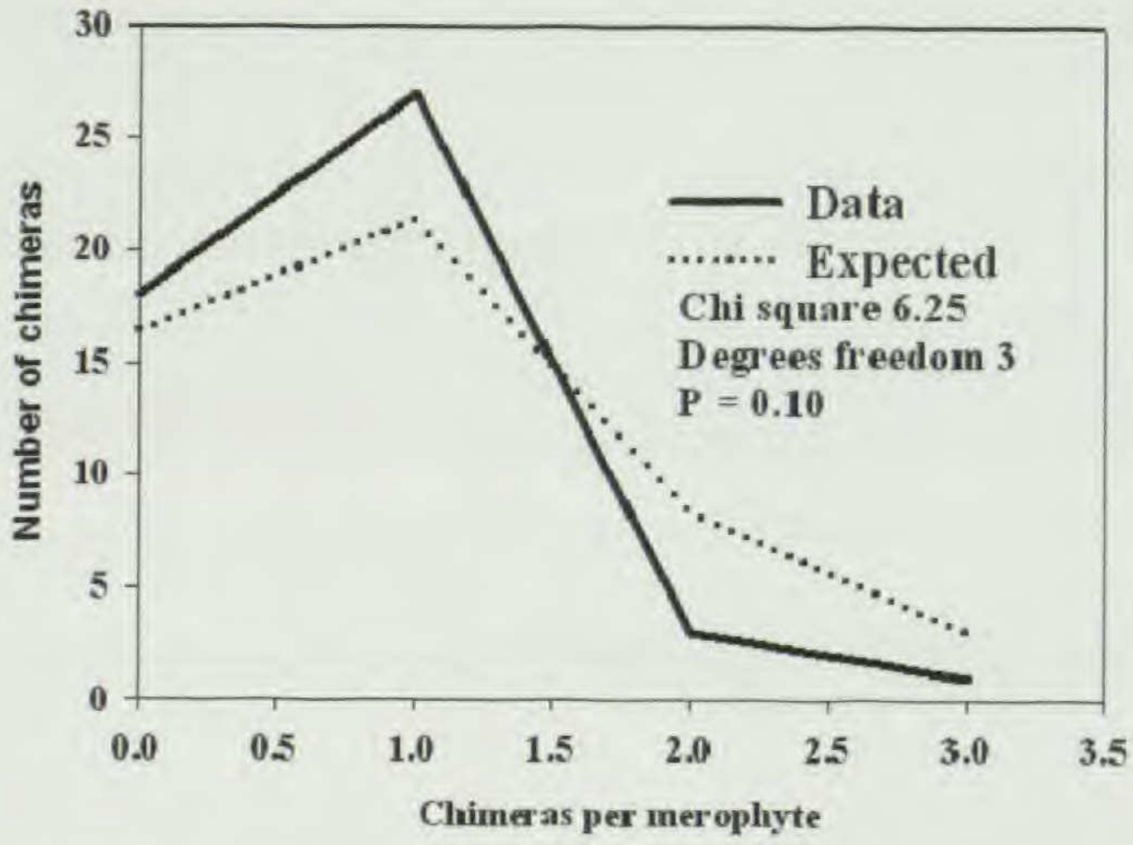


FIG. 5. Number of albino subclones per merophyte vs number of such cases.

arbitrarily taken as 0.05 at each cell generation with the initial cells passing through five cell cycles, or an increase of $2^5$-1, or 31 cells. The resultant data from five runs gave a $\chi^2$ probability of 0.10, a marginally good fit (Fig. 5). It is suggested from this statistical result that subclones arise independently of other subclones, a feature of clones demonstrated earlier for plants in general (Korn, Cell Prol. 41:691–708. 2008).

These two studies together indicate subclones form randomly among cells during the development of a merophyte. It would thus seem that all cells of the gametophyte have an unstable mutant gene for green plastids that either further spontaneously mutates or causes a non-allelic gene to mutate to an albino state. Since the gametophyte is haploid mutant genes would be expressed directly. If the plastid is the unit of expression one-celled pure subclones are not possible by immediate segregation during a cell division involving segregation of one mutant and about 15 wild type plastids randomly into daughter cells. It is not clear how the second mutation leads immediately to an albino cell but once that cell appears it proliferates into a clone of albino cells.

One unexpected feature of these 16 gametophytes is the lack of sex organ production, hence, sporophytes could not be produced. This is not unlike morphological mutations in the desmid *Cosmarium turpinii* (Korn, Genetics 65:41–49. 1970) where of 17 shape mutations 13 were sterile and only four were sexual. One interesting result is that albino subclones are smaller than green areas (Fig. 3) indicating photosynthate of cells contributes mostly to their own growth, that is, much of growth is not a property of an organism but is a local phenomenon. It is also possible that mutant subclones have too little reserves for the plant to be sexual.

Chimeras in *Dryopteris* parallels those in various species of juniper (Tilney-Bassett, Plant Chimeras, Edward Arnold. 1986; Korn, Amer. J. Bot. 88:1945–1952. 2001). In juniper the first chimera is a periclinal sandwich one with a white tunica ,WLI, and green corpus, GL2, and the second chimera is from a periclinal replacement division in L1 where one daughter is in L2 to gives rise

to a mericlinal WL2 chimera. Repetition of this type of division in the same plant generates many periclinal chimeras which are termed eversporting (Hejnowicz, 1951). Here in *Dryopteris* the first step is an unstable mutant state in all cells of the gametophyte inherited from the spore and the second step is a mutation to an albino phenotype in a cell that proliferates into an albino subclone. Repetition of this second step produces an eversporting case of mericlinal chimeras. This type of chimera involving an unstable state is too peculiar to have been anticipated by Klekowski (1984) but explains how chimeras are possible in fern gametophytes.—Robert W. Korn, Bellarmine University, 2001 Newburg Rd., Louisville, Kentucky, U.S.A.